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MAMMALIAN LYSOPHOSPHATIDIC ACID ACYLTRANSFERASE

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Technical Field of the Invention

The present invention provides polypeptides having lysophosphatidic acid acyltransferase (LPAAT) activity and polynucleotides encoding polypeptides having LPAAT activity. The present invention further provides for isolation and production of polypeptides involved in phosphatidic acid metabolism and signaling in mammalian cells, in particular, the production of purified forms of LPAAT.

Background of the Invention

LPAAT, also referred to as 1-acyl sn-glycerol-3-phosphate acyltransferase (EC 2.3.1.51), is known to catalyze the acylation of lysophosphatidic acid (LPA) to phosphatidic acid (PA) by acylating the sn-2 position of LPA with a fatty acid acyl-chain moiety. LPA and PA, while originally identified as intermediates in lipid biosynthesis (Kent, *Anal. Rev. Biochem.* 64:315-343, 1995), have more recently been identified as phospholipid signaling molecules that affect a wide range of biological responses (McPhail et al., *Proc. Natl. Acad. Sci. USA* 92:7931-7935, 1995; Williger et al., *J. Biol. Chem.* 270:29656-29659, 1995; Moolenaar, *Curr. Opin. Cell Biol.* 7:203-210, 1995).

Cellular activation in monocytic and lymphoid cells is associated with rapid upregulation of synthesis of phospholipids (PL) that includes PA, diacylglycerol (DAG) and glycan phosphatidylinositol (PI). PAs are a molecularly diverse group of phospholipid second messengers coupled to cellular activation and mitogenesis (Singer et al., *Exp. Opin. Invest. Drugs* 3:631-643, 1994). PA can be generated through hydrolysis of phosphatidylcholine (PC) (Exton, *Biochim. Biophys. Acta* 1212:26-42, 1994) or glycan PI (Eardley et al., *Science* 251:78-81, 1991; Merida et al., *DNA Cell Biol.* 12:473-479, 1993), through phosphorylation of DAG by DAG kinase (Kanoh et al., *Trends Biochem. Sci.*

15:47-50, 1990) or through acylation of LPA at the SN2 position (Bursten et al., *Am. J. Physiol.* 266:C1093-C1104, 1994).

Compounds that block PA generation and hence diminish lipid biosynthesis and the signal involved in cell activation are therefore of therapeutic interest in, for example, the areas of inflammation and oncology as well as obesity treatment. Therefore, compounds that block LPAAT activity have a similar therapeutic value.

The genes coding for LPAAT have been isolated in bacteria (Coleman, *Mol. Gen. Genet.* 232:295-303, 1992), in yeast (Nagiec et al., *J. Biol. Chem.* 268:22156-22163, 1993) and in plants (Brown et al., *Plant Mol. Biol.* 26:211-223, 1994; and Hanke et al., *Eur J. Biochem.* 232:806-810, 1995; Knutzon, et al., *Plant Physiol.* 109: 999-1006, 1995). Moreover, two human isoforms of LPAAT have been reported (West, et al., *DNA Cell Biol.* 6: 691-701, 1997). These isoforms are denominated LPAATα and LPAATβ (West, et al., *DNA Cell Biol.* 6: 691-701, 1997) and are described herein. There remains, however, a need for the isolation of additional mammalian LPAATs, which can be used, for example, to screen for compounds that inhibit LPAAT activity.

Summary of the Invention

The present invention provides cDNA sequences, polypeptide sequences, and transformed cells for producing isolated recombinant mammalian LPAAT. The present invention provides four polypeptides corresponding to human LPAAT isoforms. These polypeptides are designated hLPAATα, hLPAATβ, hLPAATγ1, hLPAATγ2, and hLPAATδ. The invention further provides fragments of these polypeptides which are biologically active, *i.e.*, which retain LPAAT activity. LPAAT activity is defined catalyzing acylation of lysophosphatidic acid (LPA) to phosphatidic acid (PA), specifically by acylating the sn-2 position of LPA with a fatty acid acyl-chain moiety.

The present invention further provides nucleic acid sequences encoding hLPAATα, hLPAATβ, hLPAATγ1, hLPAATγ2, and hLPAATδ and polynucleotides coding for biologically active fragments of hLPAATα, hLPAATβ, hLPAATγ1, hLPAATγ2, and hLPAATδ. The invention further provides "biologically active" polynucleotide fragments, which connotes polynucleotide fragments which encode polypeptides having LPAAT activity. The invention further provides purified LPAATs and antisense oligonucleotides for modulation of expression of the genes coding for

LPAAT polypeptides. Assays for screening test compounds for their ability to inhibit LPAATs are also provided.

The present invention includes the following polynucleotides coding for hLPAATα, hLPAATβ, hLPAATγ1, hLPAATγ2, and hLPAATδ. The invention provides the DNA sequences of: SEQ ID NO. 1 which encodes for hLPAATα; SEQ ID NO. 7, which encodes hLPAATβ; Figure 9, which encodes hLPAATγ1 Figure 10, which encodes hLPAATγ2; and Figure 11, which encodes and hLPAATδ.

The invention further includes the polypeptides for hLPAATα, hLPAATβ, hLPAATγ1, hLPAATγ2, and hLPAATδ, specifically, the amino acid sequences of: SEQ ID NO. 2, which represents hLPAATα; SEQ ID NO. 8, which represents hLPAATβ; Figure 9, which represents hLPAATγ1; Figure 10, which represents hLPAATγ2; and Figure 11, which represents hLPAATδ.

The invention further comprises biologically active fragments of the amino acid sequences of SEQ ID NO. 2, SEQ ID NO. 8, Figure 9, Figure 10, and Figure 11 or nucleotide fragments of SEQ ID NO. 1, SEQ ID NO. 7, Figure 9, Figure 10, and Figure 11 which encode biologically active LPAAT. The invention further includes polynucleotides which due to the degeneracy of the genetic code encode a polypeptide of SEQ ID NO. 2, SEQ. ID NO. 8, Figure 9, Figure 10, and Figure 11. The invention further includes polynucleotides capable of hybridizing to the nucleic acid sequences of SEQ ID NO. 1, SEQ ID NO. 7, Figure 9, Figure 10, and Figure 11, under high stringency conditions, and which are biologically active.

Also provided by the present invention are vectors containing a DNA sequence encoding a mammalian LPAAT enzyme in operative association with an expression control sequence. Host cells, transformed with such vectors for use in producing recombinant LPAAT, are also provided with the present invention. The inventive vectors and transformed cells are employed in a process for producing recombinant mammalian LPAAT. In this process, a cell line transformed with a DNA sequence encoding LPAAT in operative association with an expression control sequence, is cultured. The claimed process may employ a number of known cells as host cells for expression of the LPAAT polypeptide, including, for example, mammalian cells, yeast cells, insect cells and bacterial cells. The present invention further provides transformed cells that expresses active mammalian LPAAT.

The present invention further provides methods for identifying compounds that increase or decrease LPAAT activity, *i.e.*, acylation of LPA to PA. Because PA concentration is involved in numerous cellular pathways, compounds that increase or decrease acylation of LPA to PA are useful in regulating a number of cellular pathways. Such compounds can be used, for example, to augment trilineage hematopoiesis after cytoreductive therapy or to inhibit inflammation following hypoxia and reoxygenation injury (*e.g.*, sepsis, trauma, and ARDS). Moreover, the present invention contemplates the use of such compounds in an *in vitro* or *in vivo* context.

The present invention further includes: An isolated polynucleotide encoding a polypeptide having Lysophosphatidic Acid Acyltransferase (LPAAT) activity, comprising a nucleotide sequence selected from the group consisting of:

(a) the DNA sequence of Figure 9, Figure 10, or Figure 11 and biologically active fragments thereof; and

(b) a DNA sequence which encodes the polypeptide of Figure 9, Figure 10, or Figure 11 and biologically active fragments thereof.

An isolated polypeptide having LPAAT activity, comprising the amino acid sequence of Figure 9, Figure 10, or Figure 11 and biologically active fragments thereof.

A method for screening one or more compounds to determine whether the one or more compounds increases or decreases LPAAT activity, comprising:

(a) contacting the polypeptide of the present invention with one or more substrates for the polypeptide and with the one or more compounds; and

(b) measuring whether the LPAAT activity of the polypeptide is increased or decreased by the one or more compounds.

A method of expressing the polypeptide of the present invention, comprising:

(a) introducing into a cell a polynucleotide comprising a nucleotide sequence selected from the group consisting of:

(i) the DNA sequence of Figure 9, Figure 10, or Figure 11 and biologically active fragments thereof; and

(ii) a DNA sequence which encodes the polypeptide of Figure 9, Figure 10, or Figure 11 and biologically active fragments thereof,

wherein the polynucleotide is operably linked to a promoter; and

(b) maintaining or growing said cell under conditions that result in the expression of the polypeptide.

An isolated polynucleotide encoding a polypeptide having Lysophosphatidic Acid Acyltransferase (LPAAT) activity, comprising a DNA sequence capable of hybridizing under high stringency conditions to the complement of the DNA sequences, (a) or (b), described above, and which encodes a polypeptide having LPAAT activity.

Brief Description of the Drawings

Figure 1 shows the DNA sequence of the cDNA insert of pZplat.11 encoding hLPAATα.

Figure 2 shows amino acid sequence alignment of the human LPAATα coding sequence, the yeast LPAAT coding sequence, *E. coli* LPAAT coding sequence, and the maize LPAAT coding sequence. This comparison shows that human LPAATα has the greatest extended homology with yeast or *E. coli* LPAAT than with the plant LPAAT.

Figure 3 shows the DNA sequence of the cDNA insert pSP.LPAT3 encoding hLPAATβ. The nucleotide sequence analysis and restriction mapping of the cDNA clone revealed a 5' untranslated region of 39 base pairs and an open reading frame encoding a 278 amino acid polypeptide that spans positions 40-876. It also shows a 3' untranslated region of 480 base pairs from pSP.LPAT3. The initiation site for translation was localized at nucleotide positions 40-42 and fulfilled the requirement for an adequate initiation site (Kozak, *Critical Rev. Biochem. Mol. Biol.* 27:385-402, 1992).

Figure 4 shows the sequence of the hLPAATβ 278 amino acid open reading frame. The amino acid sequence was used as the query sequence to search for homologous sequences in protein databases. Search of the database based on Genbank Release 92 database from the National Center for Biotechnology Information (NCBI) using the blastp program showed that this protein was most homologous to yeast, bacterial and plant LPAATs.

Figure 5 shows amino acid sequences alignment of human LPAATβ coding sequence, human LPAATα coding sequence, yeast LPAAT coding sequence, bacterial (*E. coli*, *H. influenzae*, and *S. typhimurium*) LPAAT coding sequences, and plant (*L. douglassi* and *C. nucifera*) LPAAT coding sequences, revealing that the human LPAAT coding

sequences have a much more extended homology with the yeast or the bacterial LPAAT than with the plant LPAAT.

Figure 6 shows a comparison of LPAAT activity in A549 cells transfected with pCE9.LPAAT1 DNA, or no DNA using a TLC (thin layer chromatography) assay. These data are described in more detail in examples 3 and 4.

Figures 7 and 8 show a comparison of the production of TNF (Figure 7) and IL-6 (Figure 8) between A549 cells transfected with pCE9.LPAAT1 and control A549 cells after stimulation with IL-1β and murine TNF. These data show A549 overexpressing LPAAT produces >5 fold more TNF and >10 fold more IL-6 relative to untransfected A549 cells, suggesting that over expression of LPAAT enhances the cytokine signaling response in cells.

Figure 9 shows the DNA and the translated sequence of LPAATγ1.

Figure 10 shows the DNA and the translated sequence of LPAATγ2.

Figure 11 shows the DNA and the translated sequence of LPAATδ.

Figure 12 shows the LPAAT amino acid sequence alignment for human LPAAT γ1, γ2, and δ.

Figure 13 compares the LPAAT activity in ECV304 cells stably transfected with the expression plasmids for LPAATα (pCE9.LPAAT-α), LPAATβ (pCE9.LPAAT-β) DNA, LPAATγ1 (pC9LPTγ1), LPAATδ (pC2LPTδ), or the control vector (pCE9).

Detailed Description of the Invention

The present invention provides isolated LPAAT polypeptides and isolated polynucleotides encoding LPAAT polypeptides. The term "isolated," in this context, denotes a polypeptide or polynucleotide essentially free of other polypeptides or nucleic acid sequences, respectively, or of other contaminants normally found in nature.

The invention includes biologically active LPAAT and biologically active fragments thereof. As used herein, the term "biologically active" in the context of LPAAT activity refers to the ability to catalyze the acylation of lysophosphatidic acid (LPA) to phosphatidic acid (PA) by acylating the sn-2 position of LPA with a fatty acid acyl-chain moiety.

The term "expression product" as used throughout the specification refers to materials produced by recombinant DNA techniques.

The present invention contemplates modification of the hLPAATα, hLPAATβ, hLPAATγ1, hLPAATγ2, and hLPAATδ polypeptide sequences. Such modifications may be deliberate, as by site-directed mutagenesis, or may be spontaneous. All of the polypeptides produced by these modifications are included herein as long as the acyltransferase activity of LPAAT is present.

For example, the present invention contemplates the deletion of one or more amino acids from the polypeptide sequence of the hLPAATα, hLPAATβ, hLPAATγ1, hLPAATγ2, and hLPAATδ to create deletion variants. This deletion can be of one or more amino or carboxy terminal amino acids or one or more internal amino acids. The present invention further contemplates one or more amino acid substitutions to the polypeptide sequence of hLPAATα, hLPAATβ, hLPAATγ1, hLPAATγ2, and hLPAAT to create subsititutional variants. The present invention contemplates that such substitutional variants would contain certain functional alterations, such as stabilizing against proteolytic cleavage. Yet, it is understood that such variants retain their acyltransferase activity.

Substitutions preferably are conservative, that is, one amino acid is replaced with one of similar shape and charge. Conservative substitutions are well known in the art and include, for example, the changes of: alanine to serine; arginine to lysine; asparigine to glutamine or histidine; aspartate to glutamate; cysteine to serine; glutamine to asparigine; glutamate to aspartate; glycine to proline; histidine to asparigine or glutamine; isoleucine to leucine or valine; leucine to valine or isoleucine; lysine to arginine, glutamine, or glutamate; methionine to leucine or isoleucine; phenylalanine to tyrosine, leucine or methionine; serine to threonine; threonine to serine; tryptophan to tyrosine; tyrosine to tryptophan or phenylalanine; and valine to isoleucine or leucine.

The present invention further contemplates the insertion of one or more amino acids to the polypeptide sequences of hLPAATα, hLPAATβ, hLPAATγ1, hLPAATγ2, and hLPAATδ to create an insertional variant. Examples of such insertional variants include fusion proteins such as those used to allow rapid purification of the polypeptide and also can include hybrid polypeptides containing sequences from other proteins and polypeptides which are homologues of the inventive polypeptide. For example, an insertional variant could include portions of the amino acid sequence of the polypeptide from one species, together with portions of the homologous polypeptide from another species. Other

insertional variants can include those in which additional amino acids are introduced within the coding sequence of the polypeptides. These typically are smaller insertions than the fusion proteins described above and are introduced, for example, to disrupt a protease cleavage site.

Polypeptides of the present invention can be synthesized by such commonly used methods as t-BOC or FMOC protection of alpha-amino groups. Both methods involve step-wise syntheses whereby a single amino acid is added at each step starting from the C terminus of the peptide (Coligan et al., *Current Protocols in Immunology*, Wiley Interscience, Unit 9, 1991). In addition, polypeptide of the present invention can also be synthesized by solid phase synthesis methods (*e.g.*, Merrifield, *J. Am. Chem. Soc.* 85:2149, 1962; and Steward and Young, *Solid Phase Peptide Synthesis*, Freeman, San Francisco pp. 27-62, 1969) using copolyol (styrene-divinylbenzene) containing 0.1-1.0 mM amines/g polymer. On completion of chemical synthesis, the polypeptides can be deprotected and cleaved from the polymer by treatment with liquid HF 10% anisole for about 15-60 min at 0 °C. After evaporation of the reagents, the peptides are extracted from the polymer with 1% acetic acid solution, which is then lyophilized to yield crude material. This can normally be purified by such techniques as gel filtration of Sephadex G-15 using 5% acetic acid as a solvent. Lyophilization of appropriate fractions of the column will yield a homogeneous polypeptide or polypeptide derivatives, which are characterized by such standard techniques as amino acid analysis, thin layer chromatography, high performance liquid chromatography, ultraviolet absorption spectroscopsy, molar rotation, solubility and quantitated by solid phase Edman degradation.

The invention also provides polynucleotides which encode the hLPAAT polypeptides of the invention. As used herein, "polynucleotide" refers to a polymer of deoxyribonucleotides or ribonucleotides in the form of a separate fragment or as a component of a larger construct.

Polynucleotide sequences of the invention include DNA, RNA and cDNA sequences. Preferably, the polynucleotide sequences encoding hLPAAT are the sequences of: SEQ ID NO. 1 for hLPAATα; SEQ ID NO. 7 for LPAATβ; Figure 9 for hLPAATγ1; Figure 10 for hLPAATγ2; and Figure 11 for hLPAATδ. DNA sequences of the present invention can be obtained by several methods. For example, the DNA can be isolated using hybridization procedures which are known in the art. Such hybridization procedures

include, for example, hybridization of probes to genomic or cDNA libraries to detect shared nucleotide sequences, antibody screening of expression libraries to detect shared structural features, such as a common antigenic epitope, and synthesis by the polymerase chain reaction (PCR).

Hybridization procedures are useful for screening of recombinant clones by using labeled mixed synthetic oligonucleotide probes, wherein each probe is potentially the complete complement of a specific DNA sequence in a hybridization sample which includes a heterogeneous mixture of denatured double-stranded DNA. For such screening, hybridization is preferably performed on either single-stranded DNA or denatured double-stranded DNA. Hybridization is particularly useful for detection of cDNA clones derived from sources where an extremely low amount of mRNA sequences relating to the polypeptide of interest are present. Using stringent hybridization conditions directed to avoid non-specific binding, it is possible to allow an autoradiographic visualization of a specific cDNA clone by the hybridization of the target DNA to that single probe in the mixture, which is its complement (Wallace et al. *Nucl. Acid Res.* 9:879, 1981). Stringent conditions preferably include high stringency conditions. See, for example, Maniatis et al, *Molecular Cloning (A Laboratory Manual)*, Cold Spring Harbor Laboratory, pages 387-389, 1982. One such high stringency hybridization condition is, for example, 4 X SSC at 65 °C, followed by washing in 0.1 X SSC at 65 °C for thirty minutes. Alternatively, another high stringency hybridization condition is in 50% formamide, 4 X SSC at 42 °C.

The development of specific DNA sequences encoding hLPAAT can also be obtained by isolation of double-stranded DNA sequences from the genomic DNA, chemical manufacture of a DNA sequence to provide the necessary codons for the polypeptide of interest, and *in vitro* synthesis of a double-stranded DNA sequence by reverse transcription of mRNA isolated for a eukaryotic donor cell. In the latter case, a double-stranded DNA complement of mRNA is eventually formed which is generally referred to as cDNA. Of these three methods for developing specific DNA sequences for use in recombinant procedures, the isolation of genomic DNA isolates is the least common. This is especially true when it is desirable to obtain the microbial expression of mammalian polypeptides due to the presence of introns.

The synthesis of DNA sequences is frequently a method that is preferred when the entire sequence of amino acids residues of the desired polypeptide product is known.

When the entire sequence of amino acid residues of the desired polypeptide is not known, direct synthesis of DNA sequences is not possible and it is desirable to synthesize cDNA sequences. cDNA sequence isolation can be done, for example, by formation of plasmid- or phage-carrying cDNA libraries which are derived from reverse transcription of mRNA. mRNA is abundant in donor cells that have high levels of genetic expression. In the event of lower levels of expression, PCR techniques are preferred. When a significant portion of the amino acid sequence is known, production of labeled single or double stranded DNA or RNA probe sequences duplicating a sequence putatively present in the target cDNA may be employed in DNA/DNA hybridization procedures, carried out on cloned copies of the cDNA (denatured into a single-stranded form) (Jay et al., *Nucl. Acid Res.* 11:2325, 1983).

A cDNA expression library, such as lambda gt11, can be screened for hLPAATα, hLPAATβ, hLPAATγ1, hLPAATγ2, and hLPAATδ polypeptides using antibodies specific for hLPAATα, hLPAATβ, hLPAATγ1, hLPAATγ2, and hLPAATδ.. Such antibodies can be either polyclonally or monoclonally derived.

The polynucleotides of this invention include sequences that are degenerate as a result of the genetic code. The genetic code is described as degenerate because more than one nucleotide triplet, called a codon, can code for a single amino acid. The present invention contemplates the degeneracy of the genetic code and includes all degenerate nucleotide sequences which encode hLPAATα, hLPAATβ, hLPAATγ1, hLPAATγ2, and hLPAATδ.

The present invention also includes polynucleotide sequences complementary to the polynucleotides encoding hLPAATα, hLPAATβ, hLPAATγ1, hLPAATγ2, and hLPAATδ. Specifically, the present invention includes antisense polynucleotides. An antisense polynucleotide is a DNA or RNA molecule complementary to at least a portion of a specific mRNA molecule (Weintraub, *Sci. Amer.* 262:40, 1990). The invention embraces all antisense polynucleotides capable of inhibiting the expression of hLPAATα, hLPAATβ, hLPAATγ1, hLPAATγ2, or hLPAATδ. In a cell, the antisense polynucleotides hybridize to the corresponding mRNA, forming a double-stranded molecule. The antisense polynucleotides interfere with the translation of mRNA since the cell cannot translate mRNA that is double-stranded. Antisense oligomers of about 15 nucleotides are preferred, since they are easily synthesized and are less likely to cause

problems than larger molecules when introduced into the target of hLPAATα, hLPAATβ, hLPAATγ1, hLPAATγ2, or hLPAATδ-producing cell. The use of antisense methods to inhibit translation of genes is known (*e.g.*, Marcus-Sakura, *Anal. Biochem.* 172:289, 1988).

The present invention further includes allelic variations, i.e., naturally-occurring base changes in a species population which may or may not result in an amino acid change, to the polynucleotide sequences encoding hLPAATα, hLPAATβ, hLPAATγ1, hLPAATγ2, or hLPAATδ. The inventive polynucleotide sequences further comprise those sequences which hybridize under high stringency conditions (see, for example, Maniatis et al, *Molecular Cloning (A Laboratory Manual)*, Cold Spring Harbor Laboratory, pages 387-389, 1982) to the coding regions or to the complement of the coding regions of hLPAATα, hLPAATβ, hLPAATγ1, hLPAATγ2, or hLPAATδ. One such high stringency hybridization condition is, for example, 4 X SSC at 65 °C, followed by washing in 0.1 X SSC at 65 °C for thirty minutes. Alternatively, another high stringency hybridization condition is in 50% formamide, 4 X SSC at 42 °C.

In addition, ribozyme nucleotide sequences that cleave hLPAATα, hLPAATβ, hLPAATγ1, hLPAATγ2, and hLPAATδ are included in this invention. Ribozymes are RNA molecules possessing an ability to specifically cleave other single-stranded RNA in a manner analogous to DNA restriction endonucleases. Through the modification of nucleotide sequences which transcribe such RNAs, it is possible to engineer molecules that recognize specific nucleotide sequences in an RNA molecule and cleave it (Cech, *J. Amer. Med. Assn.* 260:3030, 1988).

There are two basic types of ribozymes, *tetrahymena*-type (Hasselhoff, *Nature* 334:585, 1988) and "hammerhead-type". *Tetrahymena*-type ribozymes recognize sequences which are four bases in length, while "hammerhead-type" ribozymes recognize base sequences 11-18 bases in length. The longer the recognition sequence, the greater the likelihood that the sequence will occur exclusively in the target mRNA species. Consequently, hammerhead-type ribozymes are preferable to *tetrahymena*-type ribozymes for inactivating a specific mRNA species.

Production of Polypeptides

Polynucleotide sequences encoding hLPAATα, hLPAATβ, hLPAATγ1, hLPAATγ2, and hLPAATδ polypeptides of the invention can be expressed in either

prokaryotes or eukaryotes. Hosts can include microbial (bacterial), yeast, insect and mammalian organisms. Methods of expressing DNA sequences inserted downstream of prokaryotic or viral regulatory sequences in prokaryotes are known in the art (Makrides, *Microbio. Rev.* 60:512, 1996). Biologically functional viral and plasmid DNA vectors capable of expression and replication in a eukaryotic host are known in the art (Cachianes, *Biotechniques* 15:255, 1993). Such vectors are used to incorporate DNA sequences of the invention. DNA sequences encoding the inventive polypeptides can be expressed *in vitro* by DNA transfer into a suitable host using known methods of transfection.

hLPAATα, hLPAATβ, hLPAATγ1, hLPAATγ2, and hLPAATδ sequences may be inserted into a recombinant expression vector. The term "recombinant expression vector" refers to a plasmid, virus or other vehicle that has been manipulated by inserting or incorporating genetic sequences. Such expression vectors contain a promoter sequence which facilitates efficient transcription of the inserted genetic sequence of the host. The expression vector typically contains an origin of replication and a promoter, as well as specific genes which allow phenotypic selection of the transformed cells. The DNA segment can be present in the vector, operably linked to regulatory elements, for example, a promoter (*e.g.*, T7, metallothionein I, or polyhedren promoters). Vectors suitable for use in the present invention include, for example, bacterial expression vectors, with bacterial promoter and ribosome binding sites, for expression in bacteria (Gold, *Meth. Enzymol.* 185:11, 1990), expression vector with animal promoter and enhancer for expression in mammalian cells (Kaufman, *Meth. Enzymol.* 185:487, 1990) and baculovirus-derived vectors for expression in insect cells (Luckow et al., *J. Virol.*67:4566, 1993).

The vector may include a phenotypically selectable marker to identify host cells which contain the expression vector. Examples of markers typically used in prokaryotic expression vectors include antibiotic resistance genes for ampicillin (β-lactamases), tetracycline and chloramphenicol (chloramphenicol acetyltransferase). Examples of such markers typically used in mammalian expression vectors include the gene for adenosine deaminase (ADA), aminoglycoside phosphotransferase (neo, G418), dihydrofolate reductase (DHFR), hygromycin-B-phosphotransferase (HPH), thymidine kinase (TK), and xanthine guanine phosphoriboseyltransferase (XGPRT, gpt).

In another preferred embodiment, the expression system used is one driven by the baculovirus polyhedrin promoter. The polynucleotide encoding LPAAT can be manipulated

by standard techniques in order to facilitate cloning into the baculovirus vector. See Ausubel *et al., supra*. A preferred baculovirus vector is the *pBlueBac* vector (Invitrogen, Sorrento, CA). The vector carrying a polynucleotide encoding LPAAT is transfected into *Spodoptera frugiperda* (Sf9) cells by standard protocols, and the cells are cultured and processed to produce the recombinant polypeptide. See Summers et al., *A Manual for Methods of Baculovirus Vectors and Insect Cell Culture Procedures*, Texas Agricultural Experimental Station.

The polynucleotides of the present invention can be expressed in any number of different recombinant DNA expression systems to generate large amounts of polypeptide. Included within the present invention are LPAAT polypeptides having native glycosylation sequences, and deglycosylated or unglycosylated polypeptides prepared by the methods described below. Examples of expression systems known to the skilled practitioner in the art include bacteria such as *E. coli*, yeast such as *Pichia pastoris*, baculovirus, and mammalian expression systems such as in Cos or CHO cells.

The polynucleotides of the present invention can be inserted into an expression vector by standard subcloning techniques. In a preferred embodiment, an *E. coli* expression vector is used which produces the recombinant protein as a fusion protein, allowing rapid affinity purification of the protein. Examples of such fusion protein expression systems are the glutathione S-transferase system (Pharmacia, Piscataway, NJ), the maltose binding protein system (NEB, Beverley, MA), the thiofusion system (Invitrogen, San Diego, CA), the Strep-tag II system (Genosys, Woodlands, TX), the FLAG system (IBI, New Haven, CT), and the 6xHis system (Qiagen, Chatsworth, CA). Some of these systems produce recombinant polypeptides bearing only a small number of additional amino acids, which are unlikely to affect the LPAAT ability of the recombinant polypeptide. For example, both the FLAG system and the 6xHis system add only short sequences, both of which are known to be poorly antigenic and which do not adversely affect folding of the polypeptide to its native conformation. Other fusion systems produce proteins where it is desirable to excise the fusion partner from the desired protein. In a preferred embodiment, the fusion partner is linked to the recombinant polypeptide by a peptide sequence containing a specific recognition sequence for a protease. Examples of suitable sequences are those recognized by the Tobacco Etch Virus protease (Life Technologies, Gaithersburg, MD) or Factor Xa (New England Biolabs, Beverley, MA) or enterokinase (Invitrogen, San Diego, CA).

In an embodiment of the present invention, the polynucleotides encoding LPAAT are analyzed to detect putative transmembrane sequences. Such sequences are typically very hydrophobic and are readily detected by the use of standard sequence analysis software, such as MacDNASIS (Hitachi, San Bruno, CA). The presence of transmembrane sequences is often deleterious when a recombinant protein is synthesized in many expression systems, especially in *E. coli*, as it leads to the production of insoluble aggregates which are difficult to renature into the native conformation of the polypeptide.

Accordingly, deletion of one or more of the transmembrane sequences may be desirable. Deletion of transmembrane sequences typically does not significantly alter the conformation or activity of the remaining polypeptide structure. However, one can determine whether deletion of one or more of the transmembrane sequences has effected the biological activity of the LPAAT protein by, for example, assaying the activity of the LPAAT protein containing one or more deleted sequences and comparing this activity to that of unmodified LPAAT. Assaying LPAAT activity can be accomplished by, for example, contacting the LPAAT protein of interest with the substrates LPA and fatty acyl-CoA and measuring the generation of PA or CoA, or, alternatively, measuring the formation of free CoA. Such assays for determining LPAAT activity are described in more detail below in the section describing screening assays.

Moreover, transmembrane sequences, being by definition embedded within a membrane, are inaccessible as antigenic determinants to a host immune system. Antibodies to these sequences will not, therefore, provide immunity to the host and, hence, little is lost in terms of generating monoclonal or polyclonal antibodies by omitting such sequences from the recombinant polypeptides of the invention. Deletion of transmembrane-encoding sequences from the polynucleotide used for expression can be achieved by standard techniques. See Ausubel *et al.*, *supra*, Chapter 8. For example, fortuitously-placed restriction enzyme sites can be used to excise the desired gene fragment, or the PCR can be used to amplify only the desired part of the gene.

Transformation of a host cell with recombinant DNA may be carried out by conventional techniques. When the host is prokaryotic, such as *E. coli*, competent cells which are capable of DNA uptake can be prepared from cells harvested after exponential growth phases and subsequently treated by a $CaCl_2$ method using standard procedures.

Alternatively, $MgCl_2$ or RbCl can be used. Transformation can also be performed after forming a protoplast of the host cell or by electroporation.

When the host is a eukaryote, methods of transfection of DNA, such as calcium phosphate co-precipitates, conventional mechanical procedures, (e.g., microinjection), electroporation, liposome-encased plasmids, or virus vectors may be used. Eukaryotic cells can also be cotransformed with DNA sequences encoding hLPAATα, hLPAATβ, hLPAATγ1, hLPAATγ2, and hLPAATδ polypeptides of the invention, and a second foreign DNA molecule encoding a selectable phenotype, such as the herpes simplex thymidine kinase gene. Another method uses a eukaryotic viral vector, such as simian virus 40 (SV40) or bovine papilloma virus to transiently infect or transform eukaryotic cells and express the hLPAATα, hLPAATβ, hLPAATγ1, hLPAATγ2, and hLPAATδ polypeptides.

Expression vectors that are suitable for production of LPAAT polypeptides preferably contain (1) prokaryotic DNA elements coding for a bacterial replication origin and an antibiotic resistance marker to provide for the growth and selection of the expression vector in a bacterial host; (2) eukaryotic DNA elements that control initiation of transcription, such as a promoter; and (3) DNA elements that control the processing of transcripts, such as a transcription termination/polyadenylation sequence. LPAAT polypeptides of the present invention preferably are expressed in eukaryotic cells, such as mammalian, insect and yeast cells. Mammalian cells are especially preferred eukaryotic hosts because mammalian cells provide suitable post-translational modifications such as glycosylation. Examples of mammalian host cells include Chinese hamster ovary cells (CHO-K1; ATCC CCL61), rat pituitary cells (GH_1; ATCC CCL82), HeLa S3 cells (ATCC CCL2.2), rat hepatoma cells (H-4-II-E; ATCC CRL1548) SV40-transformed monkey kidney cells (COS-1; ATCC CRL 1650) and murine embryonic cells (NIH-3T3; ATCC CRL 1658). For a mammalian host, the transcriptional and translational regulatory signals may be derived from viral sources, such as adenovirus, bovine papilloma virus, simian virus, or the like, in which the regulatory signals are associated with a particular gene which has a high level of expression. Suitable transcriptional and translational regulatory sequences also can be obtained from mammalian genes, such as actin, collagen, myosin, and metallothionein genes.

Transcriptional regulatory sequences include a promoter region sufficient to direct the initiation of RNA synthesis. Suitable eukaryotic promoters include the promoter of the mouse metallothionein I gene (Hamer *et al.*, *J. Molec. Appl. Genet.* 1:273,1982); the TK promoter of Herpes virus (McKnight, *Cell* 31: 355, 1982); the SV40 early promoter (Benoist et al., *Nature* 290:304, 1981); the Rous sarcoma virus promoter (Gorman et al., *Proc. Nat'l. Acad. Sci. USA* 79:6777, 1982); and the cytomegalovirus promoter (Foecking et al., *Gene* 45:101, 1980). Alternatively, a prokaryotic promoter, such as the bacteriophage T3 RNA polymerase promoter, can be used to control fusion gene expression if the prokaryotic promoter is regulated by a eukaryotic promoter (Zhou et al., *Mol. Cell. Biol.* 10:4529, 1990; Kaufman et al., *Nucl. Acids Res.* 19:4485, 1991).

An expression vector can be introduced into host cells using a variety of techniques including calcium phosphate transfection, liposome-mediated transfection, electroporation, and the like. Preferably, transfected cells are selected and propagated wherein the expression vector is stably integrated in the host cell genome to produce stable transformants. Techniques for introducing vectors into eukaryotic cells and techniques for selecting stable transformants using a dominant selectable marker are described, for example, by Ausubel and by Murray (ed.), *Gene Transfer and Expression Protocols* (Humana Press 1991). Examples of mammalian host cells include COS, BHK, 293 and CHO cells.

Purification of Recombinant Polypeptides.

The LPAAT polypeptide expressed in any of a number of different recombinant DNA expression systems can be obtained in large amounts and tested for biological activity. The recombinant bacterial cells, for example *E. coli*, are grown in any of a number of suitable media, for example LB, and the expression of the recombinant polypeptide induced by adding IPTG to the media or switching incubation to a higher temperature. After culturing the bacteria for a further period of between 2 and 24 hours, the cells are collected by centrifugation and washed to remove residual media. The bacterial cells are then lysed, for example, by disruption in a cell homogenizer and centrifuged to separate the dense inclusion bodies and cell membranes from the soluble cell components. This centrifugation can be performed under conditions whereby the dense inclusion bodies are selectively enriched by incorporation of sugars such as sucrose into the buffer and centrifugation at a selective speed. If the recombinant polypeptide is expressed in the inclusion, these can be washed in any of several solutions to remove some of the contaminating host proteins, then

solubilized in solutions containing high concentrations of urea (*e.g.*, 8 M) or chaotropic agents such as guanidine hydrochloride in the presence of reducing agents such as ß-mercaptoethanol or DTT (dithiothreitol). At this stage it may be advantageous to incubate the polypeptide for several hours under conditions suitable for the polypeptide to undergo a refolding process into a conformation which more closely resembles that of the native polypeptide. Such conditions generally include low polypeptide (concentrations less than 500 mg/ml), low levels of reducing agent, concentrations of urea less than 2 M and often the presence of reagents such as a mixture of reduced and oxidized glutathione which facilitate the interchange of disulphide bonds within the protein molecule. The refolding process can be monitored, for example, by SDS-PAGE or with antibodies which are specific for the native molecule. Following refolding, the polypeptide can then be purified further and separated from the refolding mixture by chromatography on any of several supports including ion exchange resins, gel permeation resins or on a variety of affinity columns.

Isolation and purification of host cell expressed polypeptide, or fragments thereof may be carried out by conventional means including, but not limited to, preparative chromatography and immunological separations involving monoclonal or polyclonal antibodies.

These polypeptides may be produced in a variety of ways, including via recombinant DNA techniques, to enable large scale production of pure, biologically active hLPAATα, hLPAATβ, hLPAATγ1, hLPAATγ2, and hLPAATδ useful for screening compounds for, *e.g.*, trilineage hematopoietic and anti-inflammatory therapeutic applications, and developing antibodies for therapeutic, diagnostic and research use.

Screening Assays

The hLPAATα, hLPAATβ, hLPAATγ1, hLPAATγ2, and hLPAATδ polypeptides of the present invention are also useful in a screening methodology for identifying compounds or compositions which affect cellular signaling of an inflammatory response. Such compounds or compositions to be tested can be selected from a combinatorial chemical library or any other suitable source (Hogan, Jr., *Nat. Biotechnology* 15:328, 1997).

This method comprises, for example, contacting hLPAATα, hLPAATβ, hLPAATγ1, hLPAATγ2, and/or hLPAATδ in the presence of compound and substrate for LPAAT, namely LPA and fatty acyl-CoA. These hLPAAT proteins can either be purified

prior to incubation or can be contained in extracts from a cell line or cell lines (for example, Sf9, ECV304, A549) transfected with cDNA encoding these polypeptides (West et al., *DNA Cell Biol.* 16:691, 1997). Alternatively, hLPAAT protein can be purified from transfected cells, and the protein, being a transmembrane protein, can then be reconstituted in a lipid bilayer to form liposomes for delivery into cells (Weiner, *Immunomethods* 4:201, 1994).

The effect of a compound or composition on hLPAATα, hLPAATβ, hLPAATγ1, hLPAATγ2, or hLPAATδ activity can be determined, for example, by measuring the generation of PA and CoA. PA can be measured by, for example, TLC methods described in Examples 3 and 7, found below. Alternatively, LPAAT activity can be assayed by detecting the formation of free CoA in reaction. CoA, which contains a free sulfhydryl-group, can be measured either by, for example, colorimetric or fluorescenic methods with sulfhydryl-specific reagents, such as, 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB) or ThioGlo (Covalent Associates, Woburn, MA). The observed effect on hLPAATα, hLPAATβ, hLPAATγ1, hLPAATγ2, and hLPAATδ may be either inhibitory or stimulatory.

Peptide Sequencing

Purified polypeptides prepared by the methods described above can be sequenced using methods well known in the art, for example using a gas phase peptide sequencer (Applied Biosystems, Foster City, CA). Because the proteins of the present invention may be glycosylated, it is preferred that the carbohydrate groups are removed from the proteins prior to sequencing. This can be achieved by using glycosidase enzymes. Preferably, glycosidase F (Boehringer-Mannheim, Indianapolis, IN) is used. To determine as much of the polypeptide sequence as possible, it is preferred that the polypeptides of the present invention be cleaved into smaller fragments more suitable for gas-phase sequence analysis. This can be achieved by treatment of the polypeptides with selective peptidases, and in a particularly preferred embodiment, with endoproteinase lys-C (Boehringer). The fragments so produced can be separated by reversed-phase HPLC chromatography.

Antibodies Directed to LPAAT

Antibodies to human LPAAT can be obtained using the product of an LPAAT expression vector or synthetic peptides derived from the LPAAT coding sequence coupled to a carrier (Pasnett et al., *J. Biol. Chem.* 263:1728, 1988) as an antigen. The preparation of

polyclonal antibodies is well-known to those of skill in the art. See, for example, Green et al., "Production of Polyclonal Antisera," in *Immunochemical Protocols* (Manson, ed.), pages 1-5 (Humana Press 1992). Alternatively, an LPAAT antibody of the present invention may be derived from a rodent monoclonal antibody (MAb). Rodent monoclonal antibodies to specific antigens may be obtained by methods known to those skilled in the art. *See*, for example, Kohler and Milstein, *Nature* 256:495, 1975, and Coligan *et al.* (eds.), *Current Protocols in Immunology*, 1:2.5.1-2.6.7 (John Wiley & Sons 1991). Briefly, monoclonal antibodies can be obtained by injecting mice with a composition comprising an antigen, verifying the presence of antibody production by removing a serum sample, removing the spleen to obtain B-lymphocytes, fusing the B-lymphocytes with myeloma cells to produce hybridomas, cloning the hybridomas, selecting positive clones which produce antibodies to the antigen, culturing the clones that produce antibodies to the antigen, and isolating the antibodies from the hybridoma cultures.

MAbs can be isolated and purified from hybridoma cultures by a variety of well-established techniques. Such isolation techniques include affinity chromatography with Protein-A Sepharose, size-exclusion chromatography, and ion-exchange chromatography. See, for example, Coligan at pages 2.7.1-2.7.12 and pages 2.9.1-2.9.3. Also, see Baines et al., "Purification of Immunoglobulin G (IgG)," in *Methods in Molecular Biology*, 10:79-104 Humana Press, Inc. 1992. An LPAAT antibody of the present invention may also be derived from a subhuman primate antibody. General techniques for raising therapeutically useful antibodies in baboons may be found, for example, in Goldenberg et al., international patent publication No. WO 91/11465 (1991), and in Losman et al., *Int. J. Cancer* 46:310, 1990.

Alternatively, a therapeutically useful LPAAT antibody may be derived from a "humanized" monoclonal antibody. Humanized monoclonal antibodies are produced by transferring mouse complementary determining regions from heavy and light variable chains of the mouse immunoglobulin into a human variable domain, and then, substituting human residues in the framework regions of the murine counterparts. The use of antibody components derived from humanized monoclonal antibodies obviates potential problems associated with the immunogenicity of murine constant regions. General techniques for cloning murine immunoglobulin variable domains are described, for example, by the publication of Orlandi et al., *Proc. Nat'l. Acad. Sci. USA* 86:3833, 1989. Techniques for producing humanized MAbs are described, for example, by Jones et al., *Nature* 321:522,

1986, Riechmann et al., *Nature* 332:323, 1988, Verhoeyen et al., *Science* 239:1534, 1988, Carter et al., *Proc. Nat'l Acad. Sci. USA* 89:4285, 1992, Sandhu, *Crit. Rev. Biotech.* 12: 437, 1992, and Singer et al., *J. Immun.* 150:2844, 1993, each of which is hereby incorporated by reference.

As an alternative, an LPAAT antibody of the present invention may be derived from human antibody fragments isolated from a combinatorial immunoglobulin library. See, for example, Barbas et al., *METHODS: A Companion to Methods in Enzymology* 2:119 1991, and Winter et al., *Ann. Rev. Immunol.* 12:433, 1994, which are incorporated by reference. Cloning and expression vectors that are useful for producing a human immunoglobulin phage library can be obtained, for example, from STRATAGENE Cloning Systems (La Jolla, CA). In addition, an LPAAT antibody of the present invention may be derived from a human monoclonal antibody. Such antibodies are obtained from transgenic mice that have been "engineered" to produce specific human antibodies in response to antigenic challenge. In this technique, elements of the human heavy and light chain locus are introduced into strains of mice derived from embryonic stem cell lines that contain targeted disruptions of the endogenous heavy chain and light chain loci. The transgenic mice can synthesize human antibodies specific for human antigens, and the mice can be used to produce human antibody-secreting hybridomas. Methods for obtaining human antibodies from transgenic mice are described by Green et al., *Nature Genet.* 7:13, 1994; Lonberg et al., *Nature* 368:856, 1994, and Taylor et al., *Int. Immun.* 6:579, 1994.

hLPAATα and hLPAATβ

hLPAATα

Search of the Genbank database of expressed sequence *tag* (dbest) using either the yeast or plant LPAAT protein sequences as probe came up with several short stretches of cDNA sequences with homology to the yeast or plant LPAAT protein sequence. These cDNA sequences of interest were derived from single-run partial sequencing of random human cDNA clones projects carried out by either the WashU-Merck EST or the Genexpress-Genethon program. An example of the amino acids sequence homology between the yeast LPAAT and a human cDNA clone (dbest#102250) is shown below by comparing SEQ ID NO. 3 (top amino acid sequence) with SEQ ID NO 4 (bottom amino acid sequence):

PFKKGAFHLAQQGKIPIVPVVVSNTSTLVSPKYGVFNRGCMIVRILKPISTE

* ****** * **** * * * * * ** * * **

PSNCGAFHLAVQAQVPIVPIVMSSYQDFYCKKERRFTSGQCQVRVLPPVPTE

The top line refers to the yeast LPAAT sequence from amino acids 169 to 220 and the bottom line refers to the homologous region from the dbest clone#102250. Identical amino acids between these two sequences are shown in block letters with asterisks in between

Accordingly, a synthetic oligonucleotide (o.BLPAT.2R), 5'-TGCAAGATGGAAGGCGCC-3' (SEQ ID NO. 5), was made based on the complement sequence of the conserved amino acids region, GAFHLA (SEQ ID NO. 6), of clone#102250. o.BPLAT.2R was radiolabeled at its 5'-end using γ-^{32}P-ATP and T4 polynucleotide kinase as a probe in screening a λzap human brain cDNA library (Stratagene).

Screening of the cDNA library was accomplished by filter hybridization using standard methods (*Current Protocols in Molecular Biology,* John Wiley & Sons, Inc., 1995). Duplicate filters containing DNA derived from λ phage plagues were prehybridized at 60 °C for 2 hr in 6X SSC (1 X SSC is 0.15 M NaCl, 0.015 M sodium citrate, pH 7.0), 5X Denhardt's solution (1X Denhardt's solution is 0.02% Ficoll, 0.02% bovine serum albumin, and 0.02% polyvinyl-pyrrolidone), 0.1% sodium dodecyl sulfate (SDS), 50 mg/ml sonicated and denatured salmon sperm DNA. Hybridization was carried out in the same buffer as used for prehybridzation. After hybridization, the filters were washed in 6 X SSC at 42 °C, and autoradiographed.

Of the approximately 1 X 10^6 clones from the human brain cDNA library that were screened, twelve clones were identified that hybridized with the probe in duplicate filters. Eleven out twelve clones were enriched and recovered after a secondary screen. Ten enriched phage samples were then converted to plasmid transformed cells by co-infecting *E. coli* XL1-Blue with the helper phage R408 using Stratagene's recommended procedure. Colony filter hybridization was performed and identified those colonies that "lit up" with the probe. Seven out of the ten pools of colonies contained positive clones. Two out of these seven clones, pZlpat.10 and pZlpat.11, contained inserts >2 kb. Restriction mapping using a combination of *Sst* I, *Pst* I and *Bam*HI digests showed these two clones contained many common fragments with respect to each other.

Nucleotide sequencing of the cDNA inserts in pZlpat.10 and pZlpat.11 was performed. Figure 1 shows the DNA sequence of the cDNA insert of pZplat.11. The nucleotide sequence analysis and restriction mapping of the cDNA clone revealed a 5'-untranslated region of >300 bp, an open reading frame capable of encoding a 283 amino acid polypeptide, and a 3'-untranslated region of >800 bp. The initiation site for translation was localized at nucleotide positions 319-321 and fulfilled the requirement for an adequate initiation site according to Kozak (Kozak, *Critical Rev. Biochem. Mol. Biol.* 27:385-402, 1992). There was another upstream ATG at positions 131-133 with an in-phase stop codon at positions 176-178. Except with a shorter 5'-untranslated region, the cDNA insert of pZplat.10 has the same DNA sequence as that of pZplat.11.

The sequence of the 283 amino acid open reading frame in pZplat.11 was used as the query sequence to search for homologous sequences in protein databases. Search of the database based on Genbank Release 90 from the National Center for Biotechnology Information (NCBI) using the blastp program showed that the protein encoded by pZplat.11 was most homologous to the yeast and bacterial LPAATs. Figure 2 shows amino acid sequences alignment of the putative human LPAATα coding sequence, the yeast LPAAT coding sequence, the *E. coli* LPAAT coding sequence, and the maize LPAAT coding sequence, revealing that human LPAATα has a much more extended homology with the yeast or the *E. coli* LPAAT than with the plant LPAAT.

hLPAATβ

Search of the Genbank database (Boguski, et al., *Science* 265:1993-1994, 1994) of expressed sequence tag (dbEST) using either the yeast or plant LPAAT protein sequences as probe came up with several short stretches of cDNA sequences with homology to the yeast or plant LPAAT protein sequence. These cDNA sequences of interest were derived from single-run partial sequencing of random human cDNA clones projects carried out mainly by I.M.A.G.E. Consortium [LLNL] cDNA clones program. An example of the amino acids sequence homology between the yeast LPAAT and a human cDNA clone (dbEST#363498) is shown below:

```
180         190         200         210         220         230
QQGKIPIVPVVVSNTSTLVSPKYGVFNRGCMIVRILKPISTENLTKDKIGEFAEKVRDQM
  ....:::::: :. :.. ..:    :..: ..:..:..:..:: ... ..
VRENVPIVPVVYSSFSSFYNTKKKFFTSGTVTVQVLEAIPTSGLTAADVPALRGTPATGP
          70          80          90         100         110
120
```

The top line refers to the yeast LPAAT sequence from amino acids 171 to 230 (SEQ ID NO. 9) and the bottom line refers to the homologous region from the dbest clone#363498 using the +1 reading frame (SEQ ID NO. 10). Identical and conserved amino acids between these two sequences are shown with double dots and single dot, respectively, in between. In order to find out if such cDNA clones with limited homology to yeast LPAAT sequence indeed encode human LPAATβ sequence, it was necessary to isolate the full-length cDNA clone, insert it into an expression vector, and to test if cells transformed or transfected with the cDNA expression vector produced more LPAAT activity.

Accordingly, two synthetic oligonucleotides, 5'-CCTCAAAGTG TGGATCTATC-3' (o.LPAT3.F) (SEQ ID NO. 11) and 5'-GGAAGAGTAC ACCACGGGGA C-3' (o.LPAT3.R), (SEQ ID NO. 12) were ordered (Life Technologies, Gaithersburg, MD) based on, respectively, the coding and the complement sequence of clone#363498. o.LPAT3.R was used in combination with a forward vector primer (o.sport.1), 5'- GACTCTAGCC TAGGCTTTTG C-3'(SEQ ID NO. 13) for amplification of the 5'-region, while o.LPAT3.F was used in combination with a reverse vector primer (o.sport.1R), 5'-CTAGCTTATA ATACGACTCA C-3' (SEQ ID NO. 14), for amplification of the 3'-region of potential LPAATβ sequences from a pCMV.SPORT human leukocyte cDNA library (Life Technologies, Gaithersburg, MD). A 700 bp PCR fragment derived from o.sport.1 and o.LPAT3.R amplification was cut with *EcoR* I before inserting in between the *Sma* I and *EcoR* I of pBluescript(II)SK(-) (Stratagene, LaJolla, CA) to generate pLPAT3.5'. A 900 bp PCR fragment derived from o.sport.1R and o.LPAT3.F amplification was cut with *Xba* I before inserting in between the *Sma* I and *Xba* I of pBluescript(II)SK(-) (Stratagene, LaJolla, CA) to generate pLPAT3.3'. Nucleotide sequencing analysis of the cDNA inserts from these two plasmids showed they contained overlapping sequences with each other, sequences that matched with the dbEST#363498 as well as extensive homology with the yeast LPAAT amino acids sequence (Nagiec et al., *J. Biol. Chem.* 268:22156-22163, 1993). To assemble the two

halves of the cDNA into a full-length clone, the 560 bp *Nco* I - *Nar* I fragment from pLPAT3.5' and the 780 bp *Nar* I - *Xba* I fragment from pLPAT3.3' were inserted into the *Nco* I / *Xba* I vector prepared from pSP-luc+ (Promega, Madison, WI) via a three-part ligation to generate pSP.LPAT3.

Figure 3 shows the DNA sequence ID of the cDNA insert of pSP.LPAT3. The nucleotide sequence analysis and restriction mapping of the cDNA clone revealed a 5'-untranslated region of 39 bp, an open reading frame capable of encoding a 278 amino acids polypeptide that spans nucleotide positions 40 to 876 and a 3'-untranslated region of 480 bp (Figure 3). The initiation site for translation was localized at nucleotide positions 40-42 and fulfilled the requirement for an adequate initiation site according to Kozak (Kozak, *Critical Rev. Biochem. Mol. Biol.* 27:385-402, 1992).

The sequence of the 278 amino acid open reading frame (Figure 4) was used as the query sequence to search for homologous sequences in protein databases. Search of the database based on Genbank Release 92 from the National Center for Biotechnology Information (NCBI) using the blastp program showed that this protein was most homologous to the yeast, bacterial and plant LPAATs. Figure 5 shows amino acid sequences alignment of this putative human LPAATβ coding sequence, human LPAATα coding, the yeast LPAAT coding sequence, the bacterial (*E. coli*, *H. influenzae*, and *S. typhimurium*) LPAAT coding sequences, and the plant (*L. douglassi* and *C. nucifera*) LPAAT coding sequences, revealing that the human LPAAT coding sequences have a much more extended homology with the yeast or the bacterial LPAAT than with the plant LPAAT.

<u>hLPAATγ1, hLPAATγ2, or hLPAATδ</u>

Described below is the isolation of human LPAAT isoforms hLPAATγ1, hLPAATγ2, or hLPAATδ, which are distinct from hLPAATα and hLPAATβ.

Search of the Genbank database (Boguski, et al., *Science* 265:1993-1994, 1994) of expressed sequence tag (dbEST) using the maize form-I LPAAT protein (Brown, et al., *Plant Mol. Biol.* 26: 211-223, 1994) sequences as probes resulted in the identification of several short stretches of human cDNA sequences with homology to the maize LPAAT protein sequence. These cDNA sequences of interest were derived from single-run partial sequencing of random human cDNA clones projects carried out mainly by I.M.A.G.E. Consortium [LLNL] cDNA clones program. An example of the amino acids sequence

homology between the maize LPAAT and a human cDNA clone (GenBank#T55627) is shown below:

```
150 GLQRLKDFPRPFWLALFVEGTRF 172
    :::::::::  .:: :: :::::
    GLRRLSDYPEYMWFLLYCEGTRF
```

The top line refers to the maize LPAAT sequence from amino acids 150 to 172 and the bottom line refers to the homologous region from the dbEST clone with GenBank#T55627. Identical and conserved amino acids between these two sequences are shown as double dots and single dots, respectively, in the row in between. In order to determine if these human cDNA clones with homology to maize LPAAT but distinct from human LPAATα or LPAATβ indeed encoded human LPAAT, it was undertaken to isolate the full-length cDNA clone, insert it into an expression vector, and to test if cells transformed or transfected with the cDNA expression vector produced more LPAAT activity.

Accordingly, a synthetic oligonucleotides, 5'-GACTACCCC GAGTACATG TGGTTTCTC-3' (oLPTg_1F) was ordered (Life Technologies, Gaithersburg, MD) based on the coding region corresponding to amino acids DYPEYMWFL of clone GenBank#T55627. oLPTg_1F was used in combination with a reverse vector primer (o.sport.1R), 5'-CTAGCTTATA ATACGACTCA C-3', for amplification of the 3'-region of potential LPAAT sequences from a pCMV.SPORT human leukocyte cDNA library (Life Technologies, Gaithersburg, MD). A 1,000 bp PCR fragment derived from o.sport.1R and oLPTg_1F amplification was cut with Xho I before inserting in between the Sma I and Xho I of pBluescript(II)SK(-) (Stratagene, LaJolla, CA) to generate the plasmid pLPTγ_3'. Nucleotide sequencing (performed by the Seattle Biomedical Research Institute sequencing service) analysis of the cDNA inserts from plasmid pLPTg_3'showed it contained sequences that matched with the clone GenBank#T55627 as well as extensive homology with the C-terminal end of the maize LPAAT amino acids sequence (Brown, et al., *Plant Mol. Biol.* 26: 211-223, 1994). To isolate the 5'-portion of this putative LPAAT clone, a synthetic oligonucleotide, 5'-CACATGTCCG CCTCGTACTT CTTC-3' (oLPTg_1R), complementary to a region just downstream of the Bam HI site of the cDNA within generate the plasmid pLPTg_3' was used in

combination with a forward vector primer (o.sport.1), 5'- GACTCTAGCC TAGGCTTTTG C-3' for amplification of the 5'-region from a pCMV.SPORT human leukocyte cDNA library (Life Technologies, Gaithersburg, MD). The PCR fragments generated were cut with Acc65 I and BamH I before inserting in between the Acc65 I and BamH I of pBluescript(II)SK(-) (Stratagene, LaJolla, CA). DNA sequence analysis of two cDNA clones containing, respectively, a 980 bp and a 770 bp Acc65 I - BamH I inserts showed they contained sequences that overlapped with the cDNA insert of pLPTγ_3'as well as extensive homology with the N-terminal end of the maize LPAAT amino acids sequence. The DNA sequence of these two cDNA clones diverged at the 5'-regions, suggesting the presence of two alternatively spliced variants with one variant (pLPγ1_5') containing an additional 62 amino acids at the N-terminus relative to the other one (pLPγ2_5'). To assemble the two halves of each cDNA into full-length clones, the 980 bp Acc65 I - BamH I fragment from pLPγ1_5'or the 770 bp Acc65 I - BamH I fragment from pLPγ2_5' were inserted into the Acc65 I / Xho I vector prepared from pBluescript(II)SK(-) (Stratagene, LaJolla, CA) along with the 870 bp Bam HI - Xho I fragment from pLPTγ_3'via a three-part ligation to generate pSK_LPγ1 and pSK_LPγ2, respectively.

Figure 9 shows the DNA and the translated sequence (LPAAT-γ1) of the cDNA insert of pSK_LPγ1. The nucleotide sequence analysis and restriction mapping of the cDNA clone revealed a 5'-untranslated region of 183 bp with two ATGs and an in-phase stop codon, an open reading frame capable of encoding a 376 amino acids polypeptide that spans nucleotide positions 184 to 1314 and a 3'-untranslated region of 345 bp. The initiation site for translation was localized at nucleotide positions 184-186 and fulfilled the requirement for an adequate initiation site (Kozak, *Critical Rev. Biochem. Mol. Biol.* 27:385-402, 1992).

Figure 10 shows the DNA and the translated sequence (hLPAATγ2) of the cDNA insert of pSK_LPγ2. The nucleotide sequence analysis and restriction mapping of the cDNA clone revealed a 5'-untranslated region of 232 bp with two upstream ATGs with in-phase stop codons, an open reading frame capable of encoding a 314 amino acids polypeptide that spans nucleotide positions 133 to 1177 and a 3'-untranslated region of 346 bp. The initiation site for translation was localized at nucleotide positions 233-235 and

fulfilled the requirement for an adequate initiation site (Kozak, *Critical Rev. Biochem. Mol. Biol.* 27:385-402, 1992).

The sequence of the 376 amino acid open reading frame of hLPAATγ1 (Figure 9) was used as the query sequence to search for homologous sequences in protein databases. Search of the Genbank database from the National Center for Biotechnology Information (NCBI) using the tblastn program showed that this protein was distinct but homologous to a human EST sequence with GenBank #H18562. Shown below is the amino acid sequences alignment of LPAAT-γ1 with this putative human LPAAT coding sequence (LPAAT-δ):

```
LPAAT-γ1   MGLLAFLKTQFVLHLLVGFVFVVSGLVINFVQLCTLALWPVSKQLY 46
           : :    ::.::. ::.  .::. ::::.:: .:: :: :::. :::.
LPAAT-δ    MDLAGLLKSQFLCHLVFCYVFIASGLIINTIQLFTLLLWPINKQLF 340
```

The top line refers to the human LPAAT-γ1 sequence from amino acids 1 to 46 and the bottom line refers to the homologous region from the dbEST clone with GenBank #H18562. Identical and conserved amino acids between these two sequences are shown as double dots and single dots, respectively, in the row in between. The cDNA for this putative LPAAT-δ clone (Genome Systems Inc., St. Louis, MO) was isolated for further analysis.

Figure 11 shows the DNA and the translated sequence (LPAAT-δ) of this cDNA insert. Nucleotide sequence analysis and restriction mapping revealed a 5'-untranslated region of 157 bp with an upstream ATG and stop codons in all three reading frames, an open reading frame capable of encoding a 378 amino acids polypeptide that spans nucleotide positions 158 to 1294 and a 3'-untranslated region of 480 bp. The initiation site for translation was localized at nucleotide positions 158-160 and fulfilled the requirement for an adequate initiation site (Kozak, *Critical Rev. Biochem. Mol. Biol.* 27:385-402, 1992).

Figure 12 shows the LPAAT amino acid sequence alignment from the human isoforms γ1, γ2, and δ. Amino acids identical in at least two sequences are highlighted. LPAAT-γ1 and LPAAT-δ have an overall amino acid match of 54% with respect to each other.

Example 1

This example illustrates an experiment to determine if the human LPAATα clone encodes a protein with LPAAT activity, an *E. coli* vector expressing the human LPAATα as a fusion protein with β-galactosidase was transformed into a LPAAT minus strain of *E. coli* to see if it would complement the defect in *E. coli*. Specifically, the 840 bp *Bgl* II-*Nco* I fragment, which spans the coding region of human LPAATα from amino acid 68 to beyond the stop codon, derived from pZplat.11 was inserted into a *Bgl* II / *Nco* I digested cloning vector pLitmus28 (Evans et al., *BioTechniques* 19:130-135, 1995) to generate the plasmid p28BgN. This plasmid is expected to express the human LPAATα as a fusion protein containing the first 16 amino acids of β-galactosidase and the last 216 residues of the human LPAATα coding sequence using the *lac* promoter in pLitmus28. This plasmid was transformed into the *E. coli* strain JC201 (obtained from Dr. Jack Coleman, Louisiana State University). JC201 (Coleman, *Mol. Gen. Genet.* 232:295-303, 1992; Nagiec et al., *J. Biol. Chem.* 268:22156-22163, 1993; and Brown et al., *Plant Mol. Biol.* 26:211-223, 1994) is deficient in LPAAT activity due to mutation in the plsC locus. This mutation leads to a temperature-sensitive phenotype that causes JC201 to grow slowly at 37 °C, almost not at all at 42 °C, and not at all at 44 °C. JC201 transformed with p28BgN was able to grow normally at 44 °C when compared to the wild type strain JC200 ($plsC^+$), while JC201 transformed with pLitmus28 vector was not able to support growth at 44 °C. These data suggest that the putative human LPAATα cDNA isolated here does possess LPAAT activity, as the last 216 amino acids of this cDNA is sufficient to complement the defective LPAAT gene (plsC) in JC201.

Example 2

To see if the putative human LPAATβ clone encodes a protein with LPAAT activity, an *E. coli* vector expressing this human LPAATβ as a direct product was transformed into a LPAAT minus strain of *E. coli* to see if it would complement the defect in *E. coli*. Specifically, the 1350 bp *Nco* I - *Xba* I fragment from pSP.LPAT3, which spans the entire coding region from amino acid 1 to beyond the stop codon, was inserted into a *Nco* I / *Xba* I digested cloning vector pKK388-1 (Clontech, Palo Alto, CA) to generate the plasmid pTrc.LPAT3. This plasmid was transformed into the *E. coli* strain JC201 (obtained from Dr. Jack Coleman, Louisiana State University). JC201 (Coleman, *Mol.*

Gen. Genet. 232:295-303, 1992) is deficient in LPAAT activity due to mutation in the plsC locus. This mutation leads to a temperature-sensitive phenotype that causes JC201 to grow slowly at 37 °C, almost not at all at 42 °C, and not at all at 44 °C. JC201 transformed with pTrc.LPAT3 was able to grow normally at 44 °C when compared to the wild type strain JC200 ($plsC^+$), while JC201 transformed with pKK388-1 vector was not able to support growth at 44 °C. These data suggest that the putative human LPAATβ cDNA isolated here does possess LPAAT activity, as the putative protein product of this cDNA is able to complement the defective LPAAT gene (plsC) in JC201.

Example 3

This example illustrates a group of experiments to see if overexpression of this human LPAATα would have any effect on mammalian cells. The entire cDNA insert (~2,300 bp) from pZplat.11 was cleaved with Asp718 I and Xho I for insertion into the mammalian expression vector pCE9 to generate pCE9.LPAAT1. pCE9 was derived from pCE2 with two modifications. The 550 bp BstY I fragment within the elongation factor-1a (EF-1a) intron of pCE2 was deleted. The multiple cloning region of pCE2 between the Asp718 I and BamH I site was replaced with the multiple cloning region spanning the Asp718 I and Bgl II sites from pLitmus28. The plasmid pCE2 was derived from pREP7b (Leung, et al., *Proc. Natl. Acad. Sci. USA*, 92: 4813-4817, 1995) with the RSV promoter region replaced by the CMV enhancer and the elongation factor-1a (EF-1a) promoter and intron. The CMV enhancer came from a 380 bp Xba I-Sph I fragment produced by PCR from pCEP4 (Invitrogen, San Diego, CA) using the primers 5'-GGCTCTAGAT ATTAATAGTA ATCAATTAC-3' and 5'-CCTCACGCAT GCACCATGGT AATAGC-3'. The EF-1a promoter and intron (Uetsuki, et al., *J. Biol. Chem.*, 264: 5791-5798, 1989) came from a 1200 bp Sph I-Asp718 I fragment produced by PCR from human genomic DNA using the primers 5'-GGTGCATGCG TGAGGCTCCG GTGC-3' and 5'-GTAGTTTTCA CGGTACCTGA AATGGAAG-3'. These 2 fragments were ligated into a Xba I/Asp718 I digested vector derived from pREP7b to generate pCE2.

pCE9.LPAAT1 DNA was transfected into several mammalian cell lines, including A549 cells, ECV304 cells (American Type Culture Collection, Rockville, MD), two human cell line that would produce IL-6 and TNF upon stimulation with IL-1b and murine TNF and 293-EBNA cells (Invitrogen, San Diego, CA). pCE9.LPAAT1 was digested with

BspH I before electroporating into these cell lines with a Cell-Porator™ (Life Technologies, Gaithersburg, MD) using conditions described previously (Cachianes, et al., *Biotechniques* 15:255-259, 1993). After adherence of the transfected cells 24 hours later, the cells were grown in the presence of 200 μg / ml Hygromycin B (Hyg) (Calbiochem, La Jolla, CA) to select for cells that had incorporated both plasmids. Hyg-resistant clones that expressed LPAAT mRNA at a level more than 20 fold higher relative to untransfected cells based on Northern Blot analysis (Kroczek, et al., *Anal. Biochem.* 184: 90-95, 1990) were selected for further study.

Figure 6 compares the LPAAT activity in A549 cells and in A549 cells transfected with pCE9.LPAAT1 DNA using aTLC assay. This screening assay for LPAAT activity in cell extracts was based on a fluorecent assay using fluorescent lipid substrates (Ella, et al., *Anal. Biochem.* 218: 136-142, 1994). Instead of using the PC-substrate, BPC (Molecular Probes, Eugene, OR), a synthetic PC that contains an ether linkage at the SN1 position with a fluorescent Bodipy moiety incorporated into the end of the alkyl-chain at the SN1 position, BPC was converted to Bodipy-PA using cabbage phospholipase D (Sigma, St. Louis, MO). Bodipy-PA was then converted to Bodipy-LPA using snake venom phospholipase A2. The Bodipy-LPA obtained was purified by preparative TLC for use in the LPAAT assay. The assay was carried out in total cell extracts resuspended in lysis buffer (Ella, et al., *Anal. Biochem.* 218: 136-142, 1994) supplemented with 0.5 mM ATP, 0.3 mM $MgCl_2$, 100 μM oleoyl-CoA and 10 μM Bodipy LPA. The samples were incubated for 30 min before loading onto TLC plates.

Lane 1 refers to Bodipy LPA incubated with buffer only without any cell extract added. Lane 9 refers to BPC treated with cabbage phospholipase D for generating a Bodipy-PA marker. Lanes 2 and 4 refer to Bodipy LPA incubated with control A549 cell extracts with or without lipid A, respectively. Lanes 3 and 5 refer to Bodipy LPA incubated with A549 cell extracts transfected with pCE9.LPAAT1 DNA with or without lipid A, respectively. Figure 3 shows A549 cells transfected with the LPAAT cDNA (lanes 3 and 5) contain much more LPAAT activity than those of control cells (lanes 2 and 4) as evidenced by the increased conversion of Bodipy-LPA to Bodipy-PA. Addition of lipid A to the cell extracts has little effect on LPAAT activity (lanes 2 vs 4 and 3 vs 5). A549 cell extract also contains a phosphohydrolase activity that converts Bodipy-LPA to Bodipy-monoalkylglycerol (lanes 2 to 5). Interestingly, A549 cells overexpressing

LPAAT (lanes 3 and 5) have less of this activity compared to control cells (lanes 2 and 4), suggesting this phosphohydrolase prefers LPA to PA as substrate. There is also an increase of DAG in transfected cells (lanes 3 and 5) compared to control cells (lanes 2 and 4) possibly due to partial conversion of the PA formed to DAG from this endogenous phosphohydrolase.

Example 4

To see if the expressed LPAAT cDNA clone described here would also use other glycerol-lipids that contain a free-hydroxyl group at the SN2 position, the cell extracts were incubated with the substrates NBD-lysoPC (lanes 6 and 7) and NBD-monoacylglycerol (MAG) (lanes 10 and 11) to see if there is increased conversion to lysoPC and DAG, respectively. Lane 8 and 12 refer, respectively, to NBD-lysoPC and NBD-MAG incubated with buffer only without any cell extract added. TLC analysis shows little difference in the lipid profile between the transfected and control cells (lanes 7 vs 6, lanes 11vs 10), suggesting the cloned LPAAT enzyme uses LPA as the preferred substrate. It is likely that the acyltransferases for lysoPC (Fyrst, et al., *Biochem. J.* 306:793-799, 1995) and for MAG (Bhat, et al., *Biochemistry* 34: 11237-11244, 1995) represent different enzymes from the LPAAT described here.

Example 5

pCE9.LPAAT1 DNA was transfected into A549 cells (American Type Culture Collection, Rockville, MD), a human cell line that would produce IL-6 and TNF upon stimulation with IL-1β and murine TNF. pCE9.LPAAT1 was digested with *BspH* I before electroporating into A549 cells with a Cell-Porator™ (Life Technologies, Gaithersburg, MD) using conditions described previously (Cachianes, et al., *Biotechniques* 15:255-259, 1993). After adherence of the transfected cells 24 hours later, the cells were grown in the presence of 200 µg/ml Hygromycin B (Hyg) (Calbiochem, La Jolla, CA) to select for cells that had incorporated both plasmids. A Hyg-resistant clone that expressed LPAAT mRNA at a level more than 20 fold higher relative to untransfected A549 cells based on Northern Blot analysis (Kroczek et al., *Anal. Biochem.* 184:90-95, 1990) was selected for further study.

A comparison of the production of TNF (Figure 7) and IL-6 (Figure 8) between A549 cells transfected with pCE9.LPAAT1 and control A549 cells after stimulation with IL-1β and murine TNF shows A549 overexpressing LPAAT produces >5 fold more TNF and >10 fold more IL-6 relative to untransfected A549 cells, suggesting that overexpression of LPAAT would enhance the cytokine signaling response in cells. Development of compounds that would modulate LPAAT activity should therefore be of therapeutic interest in the field of inflammation.

Example 6

Construction of pC9LPTγ1 and pC2LPTδ: The primers 5'-ggcccggtacc ATGGGCCTG CTGGCCTTC C-3' (oLPγ1_1F) and 5'-taactcCTCGAG TTATTCCTT TTTCTTAAA CTC-3' (oLPγ1_1R) were used to amplify the 1100 bp Acc65 I - XhoI fragment by PCR from the template pSK_LPg1. The fragment generated was then inserted into a Acc65 I / Xho I digested pCE9 (West, et al., *DNA Cell Biol.* 6: 691-701, 1997) expression vector to make pC9LPTγ1. Similarly, the primers 5'-atggtggtaccacc ATGGACCTC GCGGGACTG CTG-3' (oLPTδ_1F) and 5'-GGAgGATATc tAGAgGCCAC CAGTTC-3' (oLPTδ_1R) were used to amplify the 1100 bp Acc65 I - Xba I fragment by PCR from the template #H18562. The fragment generated was then inserted into a Acc65 I / Nhe I digested pCE2 (West, et al., *DNA Cell Biol.* 6: 691-701, 1997) expression vector to make pC2LPTδ.

Example 7

Expression of hLPAATγ1 and hLPAATδ in mammalian cells. Plasmids pC9LPTγ1 or pC2LPTδ were stably transfected into endothelial ECV304 cells (American Type Culture Collection, Rockville, MD). Specifically, pC9LPTγ1 or pC2LPTδ were digested with BspH I before electroporating into these cell lines with a Cell-Porator™ (Life Technologies, Gaithersburg, MD). After adherence of the transfected cells 24 hours later, the cells were grown in the presence of 500 μg / ml Hygromycin B (Hyg) (Calbiochem, La Jolla, CA) to select for cells that had incorporated plasmids. Hyg-resistant clones that expressed LPAAT-γ1 or LPAAT-δ mRNA at a level more than 10 fold higher than that of cells transfected with pCE9 or pCE2 vector, based on Northern Blot analysis, were selected for further study.

Figure 13 compares the LPAAT activity in ECV304 cells stably transfected with the expression plasmids for LPAAT-α (pCE9.LPAAT-α), LPAAT-β (pCE9.LPAAT-β) DNA, LPAAT-γ1 (pC9LPTγ1), LPAAT-δ (pC2LPTδ), or the control vector (pCE9). This screening assay for LPAAT activity in cell extracts was based on the conversion of [^{14}C]oleoyl-CoA to [^{14}C]PA using a TLC assay. The assay was carried out in total cell extracts resuspended in lysis buffer (Ella, et al., *Anal. Biochem.* 218: 136-142, 1994) supplemented with 50 μM [^{14}C]oleoyl-CoA and 200 μM LPA. The samples were incubated for 10 min, extracted from chloroform, before loading onto TLC plates. Lanes 1 and 2 refer to [^{14}C]oleoyl-CoA and LPA incubated with cell extract transfected with LPAAT-α plasmid; lanes 3 and 4, with LPAAT-β plasmid; lanes 5 and 6, with LPAAT-γ1 plasmid; lanes 7 and 8, with LPAAT-δ plasmid; and lanes 9 and 10, with control vector. ECV304 cells transfected with LPAAT-α or -β cDNA (lanes 1 to 4) contain more than 3 and 20 times, respectively, LPAAT activity when compared to those of control cells (lanes 9 and 10) as evidenced by the increased conversion of [^{14}C]oleoyl-CoA to [^{14}C]PA. Cells transfected with LPAAT-δ cDNA (lanes 7 and 8) contain about 2.5 times more LPAAT activity than those of control cells (lanes 9 and 10), whereas cells transfected with LPAAT-δ cDNA show no increase in activity when compared to those of control cells (lanes 9 and 10).

SEQUENCE LISTING

(1) GENERAL INFORMATION:
(i) APPLICANTS: Leung, David W.
Ardourel, Daniel
Hollenback, David
(ii) TITLE OF INVENTION: MAMMALIAN LYSOPHOSPHATIDIC ACID ACYL TRANSFERASE
(iii) NUMBER OF SEQUENCES: 18
(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: Cell Therapeutics, Inc.
(B) STREET: 201 Elliott Avenue West
(C) CITY: Seattle
(D) STATE: Washington
(E) COUNTRY: U.S.A.
(F) ZIP 98119
(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: 3.5" disk, 1.44Mb, double side, high density
(B) COMPUTER: PC Clone (486 microprocessor)
(C) OPERATING SYSTEM: MS-DOS Version 6.1, Windows 3.1
(D) SOFTWARE: WORD 6.0
(vi) CURRENT APPLICATION DATA :
(A) APPLICATION NUMBER:
(B) FILING DATE:
(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME:
(B) REGISTRATION NUMBER:
(C) REFERENCE/DOCKET NUMBER:
(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE:(206)282-7100
(B) TELEFAX:(206)284-6206

(2) INFORMATION FOR SEQ ID NO:1:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH:2242
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double stranded
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: cDNA to mRNA
(iii) HYPOTHETICAL: no
(iv) ANTI-SENSE: no
(v) FRAGMENT TYPE:
(vi) ORIGINAL SOURCE:
(A) ORGANISM: homo sapien
(B) STRAIN:
(C) INDIVIDUAL ISOLATE:
(D) DEVELOPMENTAL STAGE:
(E) HAPLOTYPE:
(F) TISSUE TYPE: brain
(G) CELL TYPE:
(H) CELL LINE:
(I) ORGANELLE:

(ix) FEATURE:
(A) NAME/KEY: hLPAATα
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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 718  TCT GCC GGG CTG GCC TGC TGG CTG GCA GGA GTC ATC T
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 868  AAT GGC TCC ATG CTG CCC TTC AAA CGT GGC GCC TTC C
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1093  CTC ACT GTT TTC CGG GAA ATC TCC ACT GAT GGC CGG G
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1916 ACAAGGGGAAGAAGCAGACATCAGGTGCTGCACTCACTTCTGCCCCCTGG

1966 GGAGTTGGGGAAAGGAACGAACCCTGGCTGGAGGGGATAGGAGGGCTTTT

2016 AATTTATTTCTTTTTCTGTTGAGGCTTCCCCCTCTCTGAGCCAGTTTTCA

2066 TTTCTTCCTGGTGGCATTAGCCACTCCCTGCCTCTCACTCCAGACCTGTT

2116 CCCACAACTGGGGAGGTAGGCTGGGAGCAAAAGGAGAGGGTGGGACCCAG

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(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH:283

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: polypeptide

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: NO

(v) FRAGMENT TYPE:

(vi) ORIGINAL SOURCE:

(A) ORGANISM: homo sapien

(B) STRAIN:

(C) INDIVIDUAL ISOLATE:

(D) DEVELOPMENTAL STAGE:

(E) HAPLOTYPE:

(F) TISSUE TYPE: brain

(G) CELL TYPE:

(H) CELL LINE:

(I) ORGANELLE:

(ix) FEATURE:

(A) NAME/KEY: hLPAATα

(B) LOCATION:

(C) IDENTIFICATION METHOD:

(D) OTHER INFORMATION:

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(2) INFORMATION FOR SEQ ID NO:3:
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(B) TYPE: AMINO acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: polypeptide
(iii) HYPOTHETICAL: no
(iv) ANTI-SENSE: NO
(v) FRAGMENT TYPE:
(vi) ORIGINAL SOURCE:
(A) ORGANISM: yeast
(B) STRAIN:
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(D) DEVELOPMENTAL STAGE:
(E) HAPLOTYPE:
(F) TISSUE TYPE:
(G) CELL TYPE:
(H) CELL LINE:
(I) ORGANELLE:
(ix) FEATURE:
(A) NAME/KEY: LPAAT fragment
(B) LOCATION:169-220
(C) IDENTIFICATION METHOD:
(J) PUBLICATION DATE:
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

1 PFKKGAFHLAQQGKIPIVPVVVSNTSTLVSPKYGVFNRGCMIVRILKPISTE 52

(2) INFORMATION FOR SEQ ID NO:4:
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(A) LENGTH:52
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: polypeptide
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(iv) ANTI-SENSE: no
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(B) STRAIN:
(C) INDIVIDUAL ISOLATE:
(D) DEVELOPMENTAL STAGE:
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(ix) FEATURE:
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(B) STRAIN:
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(D) DEVELOPMENTAL STAGE:
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(G) CELL TYPE:
(H) CELL LINE:
(I) ORGANELLE:
(ix) FEATURE:
(A) NAME/KEY: o.BLPAT.2R
(B) LOCATION:
(C) IDENTIFICATION METHOD:
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(2) INFORMATION FOR SEQ ID NO:6:
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(A) LENGTH:6
(B) TYPE: amino acid
(C) STRANDEDNESS: single
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(i) SEQUENCE CHARACTERISTICS:
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(B) TYPE: nucleic acid
(C) STRANDEDNESS: double stranded
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: cDNA to mRNA
(iii) HYPOTHETICAL: no
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(A) ORGANISM: homo sapien
(B) STRAIN:
(C) INDIVIDUAL ISOLATE:
(D) DEVELOPMENTAL STAGE:
(E) HAPLOTYPE:
(F) TISSUE TYPE:
(G) CELL TYPE:
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(ix) FEATURE:
(A) NAME/KEY: hLPAATβ
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

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 101 CGAGTTCTAC GCCAAGGTCG CCCTGTACTG CGCGCTGTGC TTCACGGTGT
 151 CCGCCGTGGC CTCGCTCGTC TGCCTGCTGT GCCACGGCGG CCGGACGGTG
 201 GAGAACATGA GCATCATCGG CTGGTTCGTG CGAAGCTTCA AGTACTTTTA
 251 CGGGCTCCGC TTCGAGGTGC GGGACCCGCG CAGGCTGCAG GAGGCCCGTC
 301 CCTGTGTCAT CGTCTCCAAC CACCAGAGCA TCCTGGACAT GATGGGCCTC
```

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 351 ATGGAGGTCC TTCCGGAGCG CTGCGTGCAG ATCGCCAAGC GGGAGCTGCT
 401 CTTCCTGGGG CCCGTGGGCC TCATCATGTA CCTCGGGGGC GTCTTCTTCA
 451 TCAACCGGCA GCGCTCTAGC ACTGCCATGA CAGTGATGGC CGACCTGGGC
 501 GAGCGCATGG TCAGGGAGAA CCTCAAAGTG TGGATCTATC CCGAGGGTAC
 551 TCGCAACGAC AATGGGGACC TGCTGCCTTT TAAGAAGGGC GCCTTCTACC
 601 TGGCAGTCCA GGCACAGGTG CCCATCGTCC CCGTGGTGTA CTCTTCCTTC
 651 TCCTCCTTCT ACAACACCAA GAAGAAGTTC TTCACTTCAG GAACAGTCAC
 701 AGTGCAGGTG CTGGAAGCCA TCCCCACCAG CGGCCTCACT GCGGCGGACG
 751 TCCCTGCGCT CGTGGACACC TGCCACCGGG CCATGAGGAC CACCTTCCTC
 801 CACATCTCCA AGACCCCCCA GGAGAACGGG GCCACTGCGG GGTCTGGCGT
 851 GCAGCCGGCC CAGTAGCCCA GACCACGGCA GGGCATGACC TGGGGAGGGC
 901 AGGTGGAAGC CGATGGCTGG AGGATGGGCA GAGGGGACTC CTCCCGGCTT
 951 CCAAATACCA CTCTGTCCGG CTCCCCCAGC TCTCACTCAG CCCGGGAAGC
1001 AGGAAGCCCC TTCTGTCACT GGTCTCAGAC ACAGGCCCCT GGTGTCCCCT
1051 GCAGGGGGCT CAGCTGGACC CTCCCCGGGC TCGAGGGCAG GGACTCGCGC
1101 CCACGGCACC TCTGGGNGCT GGGNTGATAA AGATGAGGCT TGCGGCTGTG
1151 GCCCGCTGGT GGGCTGAGCC ACAAGGCCCC CGATGGCCCA GGAGCAGATG
1201 GGAGGACCCC GAGGCCAGGA GTCCCAGACT CACGCACCCT GGGCCACAGG
1251 GAGCCGGGAA TCGGGGCCTG CTGCTCCTGC TGGCCTGAAG AATCTGTGGG
1301 GTCAGCACTG TACTCCGTTG CTGTTTTTTT ATAAACACAC TCTTGGAAAA
1351 AAAAAAAAAA AAAAAAAAAA AAA..1373
```

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH:274

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: polypeptide

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: NO

(v) FRAGMENT TYPE:

(vi) ORIGINAL SOURCE:

(A) ORGANISM: homo sapien

(B) STRAIN:

(C) INDIVIDUAL ISOLATE:

(D) DEVELOPMENTAL STAGE:

(E) HAPLOTYPE:

(F) TISSUE TYPE:

(G) CELL TYPE:

(H) CELL LINE:

(I) ORGANELLE:

(ix) FEATURE:

(A) NAME/KEY: hLPAATβ

(B) LOCATION:

(C) IDENTIFICATION METHOD:

(D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

```
  1 Met Glu Leu Trp Cys Leu Ala Ala Ala Leu Leu Leu Leu Leu
 16 Leu Val Gln Ser Arg Ala Ala Glu Phe Tyr Ala Lys Val Ala
 31 Leu Tyr Cys Leu Cys Phe Thr Val Ser Ala Val Ala Ser Leu
 46 Val Cys Leu Cys His Gly Gly Arg Thr Val Glu Asn Met Ser
 61 Ile Ile Gly
```

61 Trp Phe Val Arg Ser Phe Lys Tyr Phe Tyr Gly Leu Arg Phe Glu
76 Val Arg Asp Pro Arg Arg Leu Gln Glu Ala Arg Pro Cys Val Ile
91 Val Ser Asn His Gln Ser Ile Leu Asp Met Met Gly Leu Met Glu
106 Val Leu Pro Glu Arg Cys Val Gln Ile Ala Lys Arg Glu Leu Leu
121 Phe Leu Gly Pro Val Gly Leu Ile Met Tyr Leu Gly Gly Val Phe
136 Phe Ile Asn Arg Gln Arg Ser Ser Thr Ala Met Thr Val Met Ala
151 Asp Leu Gly Glu Arg Met Val Arg Glu Asn Leu Lys Val Trp Ile
166 Tyr Pro Glu Gly Thr Arg Asn Asp Asn Gly Asp Leu Leu Pro Phe
181 Lys Lys Gly Ala Phe Tyr Leu Ala Val Gln Ala Gln Val Pro Ile
196 Val Pro Val Val Tyr Ser Ser Phe Ser Ser Phe Tyr Asn Thr Lys
211 Lys Lys Phe Phe Thr Ser Gly Thr Val Thr Val Gln Val Leu Glu
226 Ala Ile Pro Thr Ser Gly Leu Thr Ala Ala Asp Val Pro Ala Leu
241 Val Asp Thr Cys His Arg Ala Met Arg Thr Thr Phe Leu His Ile
256 Ser Lys Thr Pro Gln Glu Asn Gly Ala Thr Ala Gly Ser Gly Val
271 Gln Pro Ala Gln *** 274

(2) INFORMATION FOR SEQ ID NO:9:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH:60
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: polypeptide
(iii) HYPOTHETICAL: no
(iv) ANTI-SENSE: NO
(v) FRAGMENT TYPE:
(vi) ORIGINAL SOURCE:
(A) ORGANISM: yeast
(B) STRAIN:
(C) INDIVIDUAL ISOLATE:
(D) DEVELOPMENTAL STAGE:
(E) HAPLOTYPE:
(F) TISSUE TYPE:
(G) CELL TYPE:
(H) CELL LINE:
(I) ORGANELLE:
(ix) FEATURE:
(A) NAME/KEY: LPAAT fragment
(B) LOCATION:171-230
(C) IDENTIFICATION METHOD:
(J) PUBLICATION DATE:
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

1
QQGKIPIVPVVVSNTSTLVSPKYGVFNRGCMIVRILKPISTENLTKDKIGEFAEKVRDQM
60

(2) INFORMATION FOR SEQ ID NO:10:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH:60
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: polypeptide
(iii) HYPOTHETICAL: no
(iv) ANTI-SENSE: no
(v) FRAGMENT TYPE:
(vi) ORIGINAL SOURCE:
(A) ORGANISM: homo sapien
(B) STRAIN:
(C) INDIVIDUAL ISOLATE:
(D) DEVELOPMENTAL STAGE:
(E) HAPLOTYPE:
(F) TISSUE TYPE:
(G) CELL TYPE:
(H) CELL LINE:
(I) ORGANELLE:
(ix) FEATURE:
(A) NAME/KEY: dbest clone #363498
(B) LOCATION:
(C) IDENTIFICATION METHOD:
(D) OTHER INFORMATION:
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:
1
VRENVPIVPVVYSSFSSFYNTKKKFFTSGTVTVQVLEAIPTSGLTAADVPALRGTPATGP
60

(2) INFORMATION FOR SEQ ID NO:11:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH:20
(B) TYPE: nucleotide
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: oligonucleotide fragment
(iii) HYPOTHETICAL: no
(iv) ANTI-SENSE: no
(v) FRAGMENT TYPE:
(vi) ORIGINAL SOURCE:
(A) ORGANISM:
(B) STRAIN:
(C) INDIVIDUAL ISOLATE:
(D) DEVELOPMENTAL STAGE:
(E) HAPLOTYPE:
(F) TISSUE TYPE:
(G) CELL TYPE:
(H) CELL LINE:
(I) ORGANELLE:
(ix) FEATURE:
(A) NAME/KEY: o. LPAT.3F
(B) LOCATION:
(C) IDENTIFICATION METHOD:

(D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

1 CCTCAAAGTGTGGATCTATC 20

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH:21

(B) TYPE: nucleotide

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: oligonucleotide

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: no

(v) FRAGMENT TYPE:

(vi) ORIGINAL SOURCE:

(A) ORGANISM:

(B) STRAIN:

(C) INDIVIDUAL ISOLATE:

(D) DEVELOPMENTAL STAGE:

(E) HAPLOTYPE:

(F) TISSUE TYPE:

(G) CELL TYPE:

(H) CELL LINE:

(I) ORGANELLE:

(ix) FEATURE:

(A) NAME/KEY: o.LPAT3.R

(B) LOCATION:

(C) IDENTIFICATION METHOD:

(D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

1 GGAAGAGTACACCACGGGGAC 21

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH:21

(B) TYPE: nucleotide

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: oligonucleotide

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: no

(v) FRAGMENT TYPE:

(vi) ORIGINAL SOURCE:

(A) ORGANISM:

(B) STRAIN:

(C) INDIVIDUAL ISOLATE:

(D) DEVELOPMENTAL STAGE:

(E) HAPLOTYPE:

(F) TISSUE TYPE:

(G) CELL TYPE:

(H) CELL LINE:

(I) ORGANELLE:

(ix) FEATURE:

(A) NAME/KEY: o.sport.1

(B) LOCATION:

(C) IDENTIFICATION METHOD:

(D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

1 GACTCTAGCCTAGGCTTTTGC 21

(2) INFORMATION FOR SEQ ID NO:14:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH:21
(B) TYPE: nucleotide
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: oligonucleotide
(iii) HYPOTHETICAL: no
(iv) ANTI-SENSE: no
(v) FRAGMENT TYPE:
(vi) ORIGINAL SOURCE:
(A) ORGANISM:
(B) STRAIN:
(C) INDIVIDUAL ISOLATE:
(D) DEVELOPMENTAL STAGE:
(E) HAPLOTYPE:
(F) TISSUE TYPE:
(G) CELL TYPE:
(H) CELL LINE:
(I) ORGANELLE:
(ix) FEATURE:
(A) NAME/KEY: o.sport.1R
(B) LOCATION:
(C) IDENTIFICATION METHOD:
(D) OTHER INFORMATION:
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:
1 GCTAGCTTATAATACGACTCAC 21

(2) INFORMATION FOR SEQ ID NO:15:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH:29
(B) TYPE: nucleotide
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: oligonucleotide
(iii) HYPOTHETICAL: no
(iv) ANTI-SENSE: no
(v) FRAGMENT TYPE:
(vi) ORIGINAL SOURCE:
(A) ORGANISM:
(B) STRAIN:
(C) INDIVIDUAL ISOLATE:
(D) DEVELOPMENTAL STAGE:
(E) HAPLOTYPE:
(F) TISSUE TYPE:
(G) CELL TYPE:
(H) CELL LINE:
(I) ORGANELLE:
(ix) FEATURE:
(A) NAME/KEY:
(B) LOCATION:
(C) IDENTIFICATION METHOD:
(D) OTHER INFORMATION:
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:
1 GGCTCTAGAT ATTAATAGTA ATCAATTAC 29

(2) INFORMATION FOR SEQ ID NO:16:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH:26
(B) TYPE: nucleotide
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: oligonucleotide
(iii) HYPOTHETICAL: no
(iv) ANTI-SENSE: no
(v) FRAGMENT TYPE:
(vi) ORIGINAL SOURCE:
(A) ORGANISM:
(B) STRAIN:
(C) INDIVIDUAL ISOLATE:
(D) DEVELOPMENTAL STAGE:
(E) HAPLOTYPE:
(F) TISSUE TYPE:
(G) CELL TYPE:
(H) CELL LINE:
(I) ORGANELLE:
(ix) FEATURE:
(A) NAME/KEY:
(B) LOCATION:
(C) IDENTIFICATION METHOD:
(D) OTHER INFORMATION:
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:
1 CCTCACGCAT GCACCATGGT AATAGC 26

(2) INFORMATION FOR SEQ ID NO:17:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH:24
(B) TYPE: nucleotide
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: oligonucleotide
(iii) HYPOTHETICAL: no
(iv) ANTI-SENSE: no
(v) FRAGMENT TYPE:
(vi) ORIGINAL SOURCE:
(A) ORGANISM:
(B) STRAIN:
(C) INDIVIDUAL ISOLATE:
(D) DEVELOPMENTAL STAGE:
(E) HAPLOTYPE:
(F) TISSUE TYPE:
(G) CELL TYPE:
(H) CELL LINE:
(I) ORGANELLE:
(ix) FEATURE:
(A) NAME/KEY:
(B) LOCATION:
(C) IDENTIFICATION METHOD:
(D) OTHER INFORMATION:
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:
1 GGTGCATGCG TGAGGCTCCG GTGC 24

(2) INFORMATION FOR SEQ ID NO:18:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH:28
(B) TYPE: nucleotide
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: oligonucleotide
(iii) HYPOTHETICAL: no
(iv) ANTI-SENSE: no
(v) FRAGMENT TYPE:
(vi) ORIGINAL SOURCE:
(A) ORGANISM:
(B) STRAIN:
(C) INDIVIDUAL ISOLATE:
(D) DEVELOPMENTAL STAGE:
(E) HAPLOTYPE:
(F) TISSUE TYPE:
(G) CELL TYPE:
(H) CELL LINE:
(I) ORGANELLE:
(ix) FEATURE:
(A) NAME/KEY:
(B) LOCATION:
(C) IDENTIFICATION METHOD:
(D) OTHER INFORMATION:
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:
1 GTAGTTTTCA CGGTACCTGA AATGGAAG 28